HISTOPATHOLOGICAL EFFECTS OF DIETHYL PHTHALATE ON *Clarias gariepinus* JUVENILES

IKELE, Chika Bright, MGBENKA, Bernard Obialo and OLUAH, Ndubuisi Stanley
Fisheries and Hydrobiology Research Unit, Department of Zoology, University of Nigeria, Nsukka.

Corresponding Author: Ikele, C. B. Fisheries and Hydrobiology Research Unit, Department of Zoology, University of Nigeria, Nsukka. **Email:** brightikelec@gmail.com **Phone:**

ABSTRACT

The acute toxicity of diethyl phthalate to Clarias gariepinus fingerlings was investigated using static bioassays with continuous aeration over a period of 96 h. The LD_{50} of DEP for Clarias gariepinus was determined at 95% confidence limit for log toxicant concentration after 24 h, 48 h, 72 h and 96 h. The LD_{50} after 24 h, 48 h, 72 h and 96 h were 2.22, 2.73, 3.44 and 3.93 µg/L, respectively. The LD_{50} for total death was estimated as 1.87 µg/L. During the exposure period, the test fish was restless, swam erratically and hemorrhaging of the gill filaments was evident. These observations are indicative of stress due to effect of the diethyl phthalate on the fish. Sub acute Diethyl phthalate concentrations (30 µg/L, 40 µg/L, 60 µg/L and 80 µg/L) was obtained from the LC_{50} of the acute study and the fish were exposed to the graded concentrations for period of 30 days. The fish were killed at 15 days interval and the liver and kidney were excised and the histopathological changes of DEP on the liver and kidney were determined by light microscopy. Pyknotic nuclei, destruction or fusion of tubules, condensation of the glomeruli and severe destruction of the tubule were observed in kidney tissue of fish. Cellular proliferation, congestion, necrosis, sinusoid enlargement, paranchymatous degeneration and fatty or glycogen degeneration were observed in the liver tissue of fish. The morphological changes of the tissues were dosage dependent.

Keyword: Acute and Sub-acute study, Histopathology, *Clarias gariepinus*, Diethyl phthalate

INTRODUCTION

Increasing industrial and agricultural production has resulted in increasing numbers of freshwater systems being polluted by the contaminant present in industrial wastewater releases. Most aquatic pollutants have undergone some toxicity testing to evaluate effects on non-target organism (Urban and Cook, 1986). Unfortunately these tests are rarely conducted on the early life stages of fish commonly found in water bodies in Nigeria being treated for 'weed control". The continual use and indiscriminate disposal of these pollutant has prompted some concern of the effects of this chemical on the early life stages of fish (Huang *et al*., 2008). Diethyl phthalate (DEP) is an industrial chemical used in products such insecticides, mosquito repellants, camphor substitute, plasticizer for cellulose, bathing soaps, cosmetics, pharmaceutical coatings, after shave-lotion, detergent, ester plastic film and sheets, etc (Kamrin and Mayor, 1991; Huang *et al*., 2008). Diethyl phthalate in aquatic environment originates from a variety of compounds of anthropogenic origin such as pesticides, detergents and plasticizers (Fatoki *et al.,* 2010). Many reports have discussed the impact of man-made xenoestrogenic compounds on man and wildlife (Fatoki *et al.,* 2010). Moreover, careless handling, accidental spillage, or discharge of untreated effluents into natural water-ways have harmful effects on the fish population and other forms of aquatic life and may contribute long term effects in the environment.

Fish are widely used to evaluate the health of aquatic systems, and physiological changes in fishes serve as biomarkers of environmental pollution (Winger *et al*., 1990). Thus, the objective of this study is to investigate the acute toxic effects of diethyl phthalate on *Clarias gariepinus* fingerlings and to determine the sublethal concentration with emphasis in the histopathological changes in the liver and kidney.

MATERIALS AND METHODS

One hundred and fifty fingerlings of mean weight, 13.13 ± 2.27g were obtained from Aquafish Limited, Awka, Anambra State, Nigeria, acclimated for 21 days and used for the study. The fingerlings were randomly distributed into fifteen 25 L glass containers filled to 20 L mark with aerated deep well water each containing ten fingerlings. The fish were acclimatized for three weeks before the commencement of the study. During the period of acclimation and the experiment, the fish were fed *ad libitum* on 55% crude protein diet (Oluah and Didigwu, 2008). Analytical grade diethyl phthalate (99.97% purity) obtained from Sigma Chemical, Ohio, USA was used in this study. Stock solution was prepared by dissolving 1g of DEP in 1000ml of deionized distilled water and serial dilutions made from which different concentrations (0, 50, 75, 100 and 150 µg/L) corresponding to the treatments were made.

Lethality of *Clarias gariepinus* in Diethyl phthalate: A static bioassay technique (ASTM 729-90) (ASTM, 1990) was adopted and preliminary screening was carried out to determine the appropriate lethal concentration of DEP (Solbe, 1995). Five treatments with six DEP concentrations i.e. 50, 75, 100 and 150 µg/L and a control with no DEP were conducted in triplicate tanks. The cumulative mortality was recorded for 24 – 96 h and fish were examined to determine the cause of death. Immediate behavioural changes of the fish were recorded before death. Water quality characteristics of temperature, pH, dissolved oxygen (DO) and total hardness as equivalent of calcium carbonate were determined. The temperature was 27.1°C, pH 7.9, DO 6.2 mg/L and total hardness 100 mg/L equivalent of $CaCO_3$.

Sublethal Toxicity: In this study, 180 fingerlings of *Clarias gariepinus* (13.13 ± 2.27 g) were exposed to sublethal concentrations of DEP in water (30, 40, 60 and 80 µg/L). The temperature, pH and the dissolved oxygen of the tap water used in the study were 27.5° C, 7.2 mg/L and 6.4 mg/L respectively while the total hardness was 100 mg/L $CaCO_3$. The fish were randomly divided into five treatments groups (A – E) of thirty six fish. Each group was further randomized into three replicate experiments containing twelve fish each. Experiments were run under static renewal bioassay for 0 – 30 days. After each of the exposure periods of 0, 15 and 30 day, respectively, fish from the experimental groups as well as control aquaria were sacrificed and the gills collected and fixed in 10% formal saline, processed routinely, embedded in paraffin, sectioned at 6 µm thickness, stained with heamatoxylin and eosin (H&E), and examined using light microscopy (NIKON TE 3000). Photomicrographs were taken at x10, x20, or x40 magnification with a digital camera (Nikon 9000).

Analysis: The lethal concentration (LC_{50}) at 96 h was computed using the probit analysis. Statistical differences among treatment means was conducted using analysis of variance (ANOVA) with post hoc F-LSD.

RESULTS

Toxicity of Diethyl phthalate: The LC_{50} was based on probit analysis was found to be 1.87 µg/L and 3.93 µg/L at log toxicant concentration for total death and 96 h exposure respectively as shown in Figures 1 and 2. The LD_{50} values for DEP were 2.22, 2.73, 3.44 and 3.93 µg/L after 24, 48 and 72 h, respectively. The r^2 value was 97.6% indicating the adequacy of the model. No adverse behavioral changes or mortality was recorded in the control fish throughout the period of the bioassay. The behavior of the control fish and their colour were normal.

Symptomatic behavioural toxicosis observed in DEP treated fishes were; lack of balance, agitated or erratic swimming, air gulping, restlessness, sudden quick movement and rapid opercula movement. *C. gariepinus* normal darkly pigmentation in the dorsal and lateral parts was changed to very light pigmentation in the dorsal and lateral parts.

Histopathological Changes

Liver: The histology of control fish liver revealed normal typical paranchymatous appearance (Figure 3). The liver was made up of hepatocytes that were polygonal cells with a central spherical nucleus and a densely stained nucleolus. Congestion of central vein (40 µg/L, day 30, Figure 4), severe cellular proliferation (30 µg/L and 40 µg/L, day 15 and 30, Figure 5), severe necrosis sinusoid enlargement and (60 µg/L, day 15 and 30 Figure 6) and parachymatous degeneration and fatty/glycogen degeneration (80 µg/L, day 15 and 30 Figure 7).

Kidney: No recognizable changes were observed in the kidney of the control fish. At light microscopy level, the renal corpuscle was composed of the glomerulus and Bowman's capsule. No changes were seen in control and group A treated with 30 µg/l as shown in Figures 8, 9 and 10. The kidney tissue from *C. gariepinus* exposed to different concentration of DEP showed degenerated kidney pykinosis, condensation of glomeruli content and accumulation of hyaline droplets in the tubular epithelial cells as shown in Figures 10, 11, 12, 13, 14, 15 and 16, respectively. The mortality of *Clarias gariepinus* fingerlings on 24 – 96 hours exposure to varied concentrations of diethyl phthalate was concentration and time dependent (Table 1).

DISCUSSION

Diethyl phthalate is used in pharmaceutical coating as a fixative in cosmetics, in the manufacture of celluloid, as solvent for cellulose acetate in the manufacture of varnishes and ropes in the denaturation of alcohol, perfume binders (Sonde *et al.*, 2000). Because DEP has being used extensively for various purposes, contamination of the environment by DEP cannot be ruled out. Results obtained from this study showed that the mortality of *Clarias gariepinus* fingerlings increased with increase concentration of diethyl phthalate and was dose dependent. The rapid opercula movement, erratic swimming and loss of balance observed in this study suggested possible nervous disorder. Haemorrhaging of the gill when the test fish were exposed to 100 µg/L and 150 µg/L of the compound is indicative of toxicity of the chemical. This is probably due to rupture of blood vessels of the gills and possible reduction in the haemotological parameters of erythrocyte count, haematocrit and mean corpuscular volume of the fish. The LD_{50} reported in this study is less than the observed field concentration in the water column (0.16 - 3.53 mg/L)and sediment (0.16 - 0.32 mg/L) of DEP in the Venda region of South African waters (Fatoki *et al.*, 2010) where similar indiscriminate discharge of DEP-laden effluents and wastes take place as in Nigeria. In Nigeria, there is dearth of information about the field levels of DEP but it is expected to be higher than the LD_{50} reported in the present study. In the present study, the liver of *C. gariepinus* exposed to DEP concentration showed congestion (cirrhosis), reduction of filament and enlargement of sinusoid and necrosis. Liver is especially useful organ in assessing the possible impact of pollutant in fish. This is because chemical tends to concentrate there. This is also a major site for biotransformation of toxic chemicals which usually makes them less toxic and more easily excreted. In the study of Risbourg and Bastide (1995), the exposure of fish to atrazine herbicide increased in the size of lipid droplets, vacuolization in the liver. The most frequent encountered types of degenerative changes are those of hydropic degeneration, cloudy swelling, vacuolization and focal necrosis. This also agrees with Babu *et al.* (2007) in the exposure of fish to fenevalerate on the liver tissues of *Cirrhina mrigala*, when necrosis of tubular epithelium and pycnotic nuclei in the haematopoietic tissue occurred. Necrosis of the liver tissues in the study was observed, probably resulted from the excessive

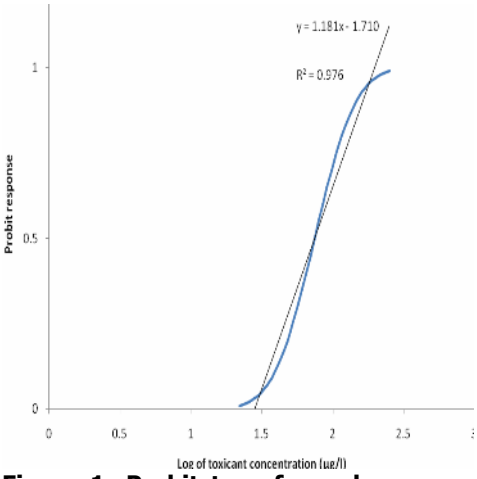

Figure 1: Probit transformed responses for total death of *Clarias gariepinus* fingerlings exposed to graded concentrations of diethyl phthalate

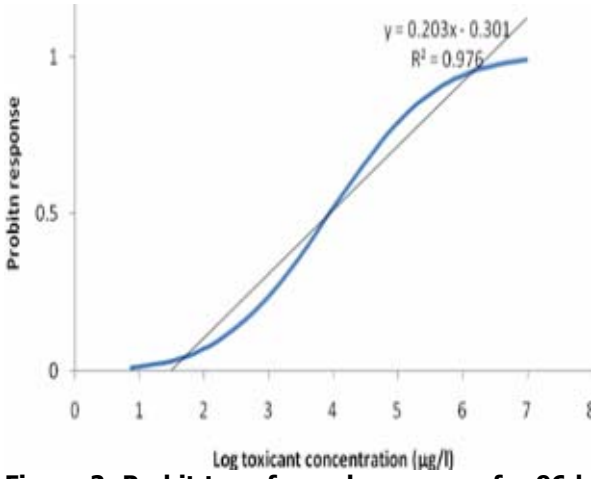

Figure 2: Probit transformed responses for 96 h exposure of *Clarias gariepinus* fingerlings exposed to graded concentrations of diethyl phthalate

Table 1: Mortality of *Clarias gariepinus* fingerlings on 24 – 96 hours exposure to varied concentrations of diethyl phthalate

Toxicant concentration (µg/l)	Log concentration	Exposure time (h) 24	48	72	96	Total mortality	Total Number survived
50	1.69	0	2	1	1	4	6
		1	1	1	0	3	7
		0	1	1	0	2	8
75	1.87	1	2	1	1	5	5
		0	2	2	0	4	6
		1	1	1	2	5	5
100	2	2	3	1	0	6	4
		2	2	1	0	5	5
		3	2	0	1	6	4
150	2.17	6	2	1	1	10	0
		3	3	3	1	10	0
		4	3	2	1	10	0
Control	0	0	0	0	0	0	10
		0	0	0	0	0	10
		0	0	0	0	0	10

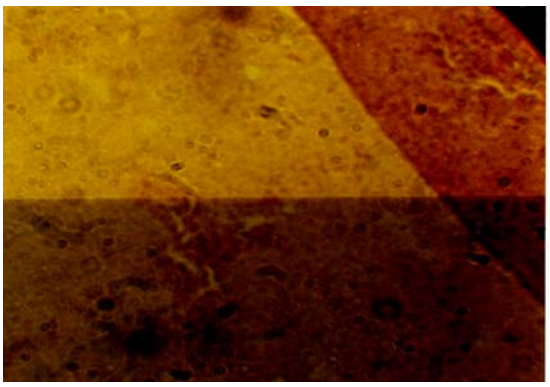

Figure 3: Liver section of group A exposed to 30 µg/l diethyl phthalate for 15 days, Liver exhibits normal morphology. (H&E) Mag. x 40

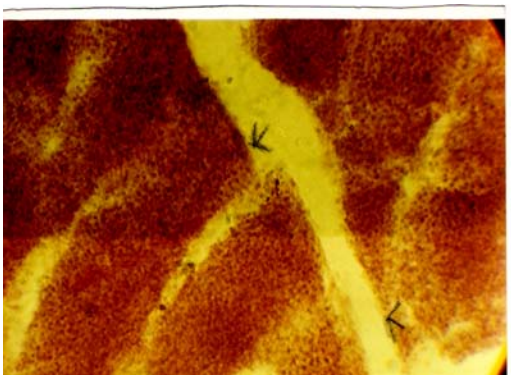

Figure 4: Liver section of group B exposed to 40 µg/l diethyl phthalate for 30 days, Liver exhibits congestion. (H&E) Mag. x 40

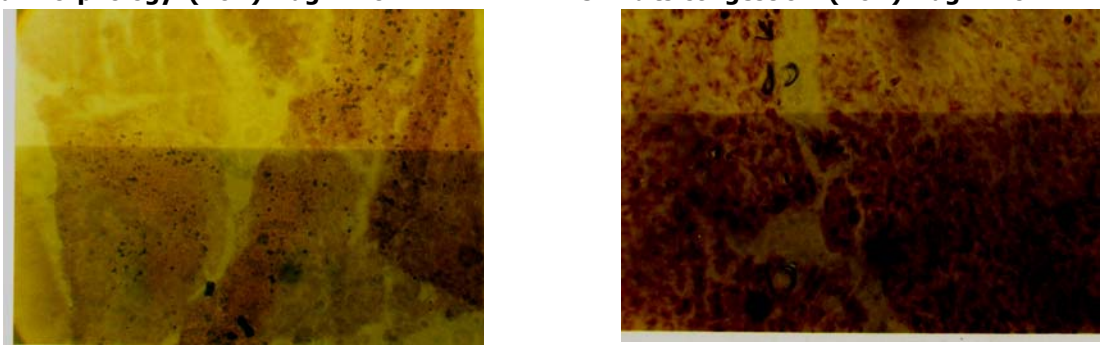

Figure 5: Liver section of group A exposed to 30 µg/L and 40 µg/L diethyl phthalate for 15 and 30 days Cellular proliferation. (H&E) Mag. x40

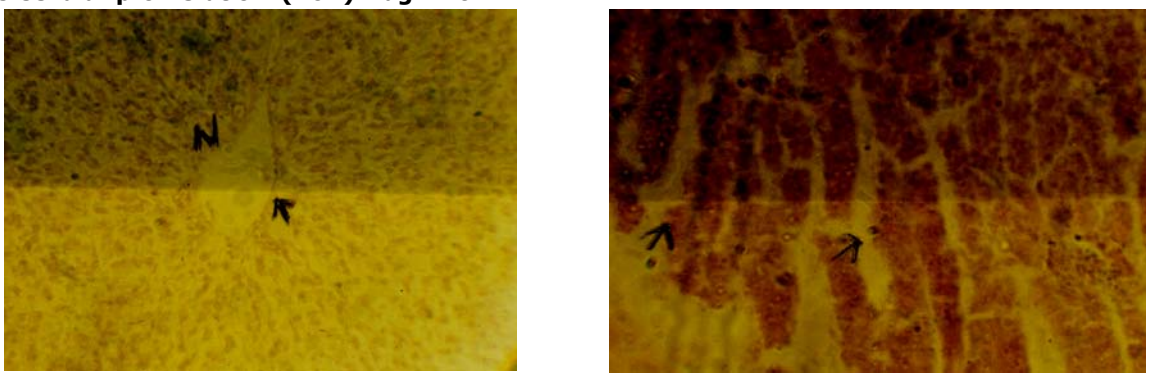

Figure 6: Liver section of group C exposed to 60 µg/l diethyl phthalate for 15 and 30 days. Necrosis of liver and Sinusoid enlargement. (H&E) Mag. x40

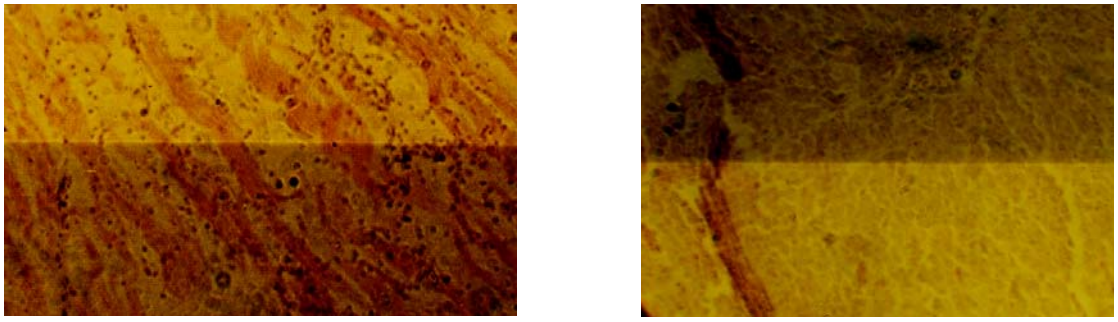

Figure 7: Liver section of group D exposed to 80 µg/l diethyl phthalate for 15 and 30 days Paranchymateous degeneration and fatty/glycogen degeneration. (H&E) Mag. x40

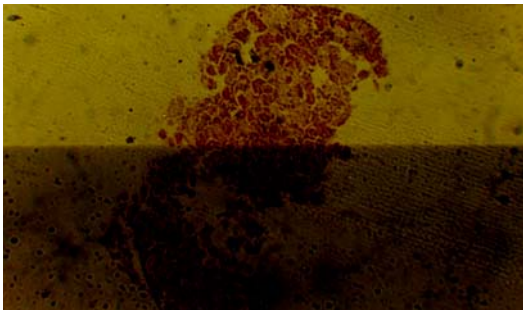

Figure 8: No recognizable changes were observed in the kidney of control fish. (H&E) Mag. x40

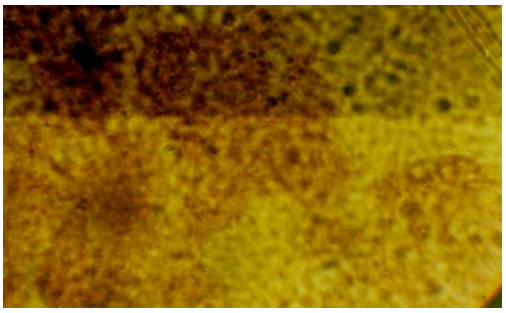

Figure 9: Kidney section of group A exposed to 30 µg/l diethyl phthalate for 15 days. Tubules are intact. (H&E) Mag. x40

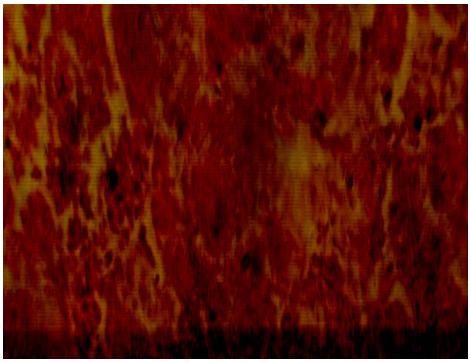

Figure 10: Kidney section of group A exposed to 30µg/l diethyl phthalate for 30 days. Tubules are intact (CS) and sequensiation of kidney architecture. (H&E) Mag. x40

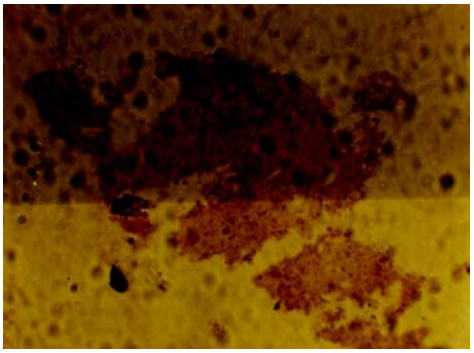

Figure 11: Kidney section of group B exposed to 40µg/l diethyl phthalate for 15 days Destruction or fusion of the tubules. (H&E) Mag. x40

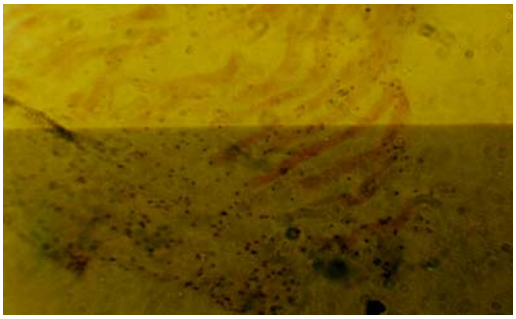

Figure 12: Kidney section of group B exposed to 40µg/l diethyl phthalate for 30 days. Severe destruction of the tubules. (H&E) Mag. x40

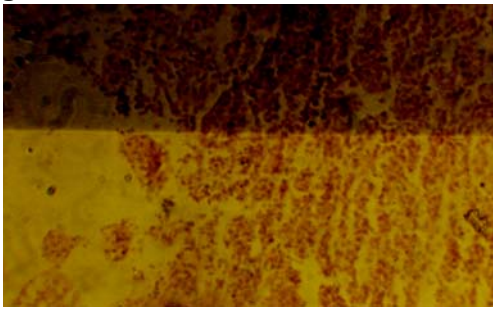

Figure 13: Kidney section of group C exposed to 60µg/l diethyl phthalate for 15 days. Pyknosis (pyvnotic nuclei present, degenerated kidney tubule pyknosis. (H&E) Mag. x40

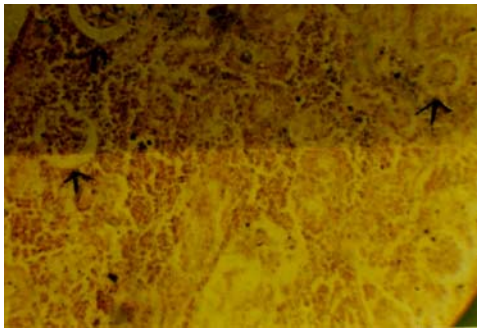

Figure 14: Kidney section of group C exposed to 60 µg/l diethyl phthalate for 30 days. Destruction of tubule, Tubules are not continuous. (H&E) Mag. x40

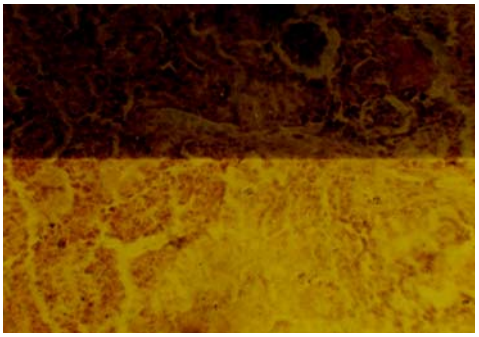

Figure 15: Kidney section of group D exposed to 80 µg/l diethyl phthalate for 15 days. Condensation of the glomeruli content. (H&E) Mag. x40

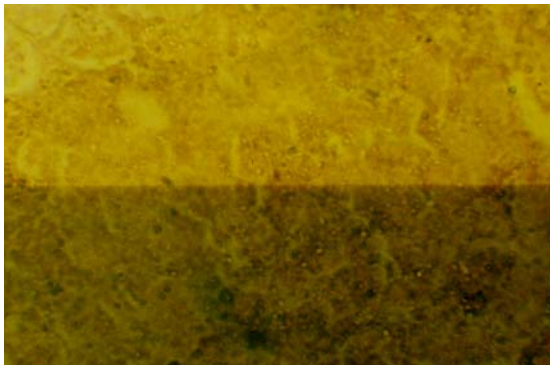

Figure 16: Kidney section of group D exposed to 80 µg/l diethyl phthalate for 30 days. Condensation of the glomeruli content. (H&E) Mag. x40

work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. The inability of fish to regenerate new liver cells may also have led to necrosis. Neskovic *et al.* (1996) conducted sub lethal toxicity test (14 days) of sub lethal glyphosate concentration on histopathological changes of carp organ such as gill, liver and kidneys. In the present study the kidney of *Clarias gariepinus* fingerlings exposed to DEP graded concentration showed tubular destruction or fusion of the tubules, pyknosis, condensation of glomeruli content and accumulation of hyaline droplets in the tubular epithelial cells. Oulmi *et al*. (1995) studied the effect of linuron herbicide on the rainbow trout (*Oncorhynchus mykiss*).Their results showed small cytoplasmic vacuoles, nuclear deformation in the epithelium of the first and second segments of the proximal tubule. The kidney cells (hepatocytes) were observed to have been massively destroyed. The renal corpuscles of the kidney were scattered resulting in the disorganization and consequently obstruction to their physiological function. Some of the kidney cells were found clogging together while they were disintegrated in some tissues of the organ. Similar findings had earlier been reported by Omoniyi *et al*. (2002) and Rahman *et al*. (2002). Lesions in the kidney tissues of fish exposed to deltamethrin had necrosis of the epithelial cells of renal tubule, pyknotic nuclei in the haematopoietic tissues, dilution of glomerular capillaries and degeneration of glomerulus (Elif, 2006). It can be concluded that DEP was toxic to fish and causes histopathological changes in fish organs. *C. gariepinus* are more susceptible to pollutant; therefore their use on/near fish farm or in area close to aquatic environment should be discouraged.

REFERENCES

ASTM (1990). *Guide for Conducting Acute Toxicity Test Fishes, Macroinvertebrates and Amphibians.* American Society for Testing of Materials (ASTM) 729 – 790.

BABU, V., MARIADOSS, S., ELLIF, C. I. and ERHAN, U. (2007). Effect of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala. Journal of Environmental Science and Health*, 42(2): 157 – 163.

ELIF, I. C. (2006). Gill and Kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. *Environmental Toxicology and Pharmacology,* 22(2): 200 – 204.

FATOKI, O. S., BORNMAN, M., RAVANDHALALA, L., CHIMUKA, L. GENTHE, B. and ADENIYI, A. (2010). Phthalate plasticizers in freshwater systems of Venda, South Africa and potential health effects. *Water South Africa,* 36(1): 117 – 125.

HUANG, P. C., TIEN, C. J., SUN, Y. M., HSIEH, C. Y. and LEE, C. C. (2008). Occurrence of phthalates in sediment and biota: Relationship to aquatic factors and the biota-sediment accumulation factor. *Chemosphere,* 73: 539-544.

KAMRIN, M. A. and MAYOR, G. W. (1991). Diethyl phthalate – a perspective. *Journal of Clinical Pharmacology*, 31(5): 484 – 489.

NESKOVIC, N. K., POLEKSIC, V., ELEZOVIC, I. and BUDIMIR, M. (1996). Biochemical and histopathological effects of glyphosate on carp *Cyprinus carpio. Bulletin of Environment Contamination Toxicology,* 56: 295 – 302.

OLUAH, N. S. and DIDIGWU, I. M. (2008). Tissue glucose and haemoglobin levels

in the catfish *Clarias albopunctatus* during anaesthesia with ketamine hydrochloride. *Animal Research International*, 5(3): 904 – 907.

OMONIYI, I., AGBON, A. O. and SODENKE, S. A. (2002). Effect of lethal and Sub lethal concentration of tobacco (*Nicotiana tobaccum*) leaf dust extract on weight and haematological changes in *Clarias garrepinus* (Burchell). *Journal of Applied Science and Environmental Management,* 6(2): 37 – 41.

OULMI, Y., NEGELE, R. D. and BRAUNBEEK, T. (1995). Cytopathology of liver and kidney in rainbow trout (*Oncorhynchus mykiss*) after long-term exposure to sub lethal concentrations of linuron. *Aquatic Toxicology*, 21: 35 – 52.

RAHMAN, M. Z., HOSSAIN, Z., MELLAH, M. F. R. and AHMED, G. U. (2002). Effect of Diazinon 60EC on *Anabus testudinus, Channa punctatus* and *Barbades gomonotus*. *NAGA The ICLARM Quarterly,* 25: 8 – 11.

RISBOURG, S. B. and BASTIDE, J. (1995). Hepatic Pertubation induced by a herbicide (atrazine) in Juvenile grey mullet *Liza ramada:* An ultrastructural study. *Aquatic Toxicology*, 31: 217 – 219.

SOLBE, J. F. (1995). Freshwater. Pages xxx – aaa *In:* CALINS, P. *Handbook of Ecotoxicology* Blackwell Science Limited. Oxford, OX 20EL.

SONDE, V. D., SCOUVA, A., TARAPOME, R., KHARE. M. P., STRIKAR, P. and RAO, C. V. (2000). Simultaneous administration of diethyl phthalate and ethyl alcohol and its toxicity in male Spranque-Dewely rats. *Toxicology,* 147: 23 – 31.

URBAN, O. J. and COOK, N. J. (1986). Hazard evaluation division, standard evaluation procedure, ecological risk assessment. EPA 540/9-85-001. Final report, U.S. Environmental Protection Agency, Washington, DC.

WINGER, P. V., SCHULTZ, D. P. and JOHNSON, W. W. (1990). Environmental contaminant concentrations in Lower savannah River, Georgia and South Carolina. *Archives of Environmental Contamination and Toxicology,* 19: 101 – 117.